

## RESEARCH NOTE

# New Record of three *Penicillium* species isolated from the Freshwater Environment in Korea

Hye Yeon Mun<sup>\*</sup>, Yu Jeong Jeon, and Jaeduk Goh

Fungi Research Team, Microbial Research Department, Nakdonggang National Institute of Biological Resources, Sangju 37242, Korea

\*Corresponding author: outcastm@nnibr.re.kr

## ABSTRACT

In this study, we aimed to observe the diversity of aquatic fungi by collecting deposits of soil, plants, and plant litter from streams and rivers. Three *Penicillium* strains were isolated from soil in streams. Based on the morphological characteristics and phylogenetic analysis using DNA sequences of the internal transcribed spacer,  $\beta$ -tubulin and calmodulin genes, the isolates were identified as *Penicillium guanacastense*, *P. saturniforme*, and *P. scabrosum*. These three fungi have not yet been reported in Korea.

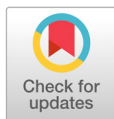
**Keywords:** Freshwater environment, *Penicillium guanacastense*, *P. saturniforme*, *P. scabrosum*

The freshwater environment consists of various diverse microorganisms and provides plenty of habitat for fungi, such as plant litter, soil, and freshwater plants. In the environment, fungi play a role in controlling nutrient and carbon cycles as a decomposer [1].

The genus *Penicillium* belongs phylogenetically to Trichocomaceae, and the name is originated from penicillus, little brush by Link. *Penicillium* is a ubiquitous and widespread fungi, which can live in any environment such as soil, air, indoor and various foods, and produce beneficial secondary metabolite [2]. To our knowledge, over 100 species of *Penicillium* have been reported in Korea [3]. Recently, *P. piscarium* from Seungcheon reservoir and *P. pasqualens* and *P. sanguifluum* from Dokdo have been reported in Korea [3,4].

In this study, we isolated three *Penicillium* species from the freshwater environment in Korea. Through molecular phylogenetic analysis and morphological characterization, the three fungal strains were identified as *Penicillium guanacastense*, *P. saturniforme*, and *P. scabrosum*. Here, we present their mycological descriptions and phylogenetic relationships.

Fungal strains were collected from soil underwater at Namsaengi-mot in Jeju, Soyang-river in Chuncheon-si and Gamcheon in Gimcheon-si between 2016-2018 (Table 1). Soil samples were treated using the dilution plate method and then incubated at 20°C. Three-point inoculation was performed on potato dextrose agar (PDA; Difco, BD, Franklin Lakes, NJ, USA), Czapek yeast autolysate agar (CYA; Czapek concentrate 10 mL, sucrose 30 g, yeast extract 5 g, K<sub>2</sub>HPO<sub>4</sub> 1 g, trace elements stock solution 1 mL,



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agar 20 g, dH<sub>2</sub>O 1 L), yeast extract sucrose agar (YES; yeast extract 20 g, sucrose 150 g, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.5 g, trace element stock solution 1 mL, agar 20 g, dH<sub>2</sub>O 885 mL) and creatine sucrose agar (CREA; sucrose 30 g, creatine·1H<sub>2</sub>O 3 g, K<sub>3</sub>PO<sub>4</sub>·7H<sub>2</sub>O, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.5 g, KCl 0.5 g, FeSO<sub>4</sub>·7H<sub>2</sub>O 0.01 g, bromocresol purple 0.05 g, trace element stock solution 1 mL, agar 20 g, dH<sub>2</sub>O 1 L) to observe the morphological characters of the isolated strains [2]. Colony diameter was measured after 7 days of inoculation, and morphological characteristics were observed using a model Eclipse Ni-U microscope (Nikon, Tokyo, Japan).

**Table 1.** Environmental factors of sampling site.

Site	Collection date	Location	GPS
Namsaengi-mot	2016.08.09	Jeju island	N33°31'57.7"E126°36'51.7"
Gamcheon	2016.03.23	Gimcheon-si, Gyeongsongbuk-do	N35°53'33.3"E127°59'2.6"
Soyang-river	2018.03.09	Chuncheon-si, Gangwon-do	N37°59'01.0"N127°49'05.0"E

Fungal genomic DNA was isolated using the NucleoSpin Plant II DNA extraction Kit (Macherey-Nagel, Duren, Germany). For the identification of the fungi, amplification of an internal transcribed spacer (ITS) region using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'),  $\beta$ -tubulin gene using Bt2a (5'-GGTAACCAAATCGGTGCTGCTTTTCG-3') and Bt2b (5'-ACCCTCAGTGTAGTGACCCTTGGCG-3') and calmodulin gene using cmd5 (5'-CCGAGTACAAGGARGCCTTC-3') and cmd6 (5'-CCGATRGAGGTCAATRACGTGG-3') were performed [5]. Similarity searches of the DNA sequences were carried out using BLASTn algorithms available at the National Center for Biotechnology Information (NCBI). For the phylogenetic analysis, MEGA7 software was used [6]. A phylogenetic tree was constructed by the neighbor-joining (NJ) method with 1,000 bootstrap replications. Reference sequences of other fungi were obtained from GenBank at NCBI (Table 2).

### Taxonomy

*Penicillium guanacastense* K.G. Rivera, Urb & Seifert, Mycotaxon 119, 315-28 (2012) [7] (Fig. 1 and 2).

**Mycobank No.:** MB563044

### Description:

**Colonies on PDA** texture floccose to granular, slight radial sulcate, front olive green to dark green, reverse dark brown to orange, 4.5 mm on 7 days at 25°C; **YES** texture velutinous to fasciculate, sulcate, some yellow mycelium, front dark green to pale grayish green, reverse light green to light yellow, 5 mm on 7 days at 25°C; **CYA** texture velutinous, exudate present orange droplet, radial sulcate, front center white, middle whitish green, margin light lemon, reverse dark to light orange, 4 mm on 7 days at 25°C; **CREA** high acid production.

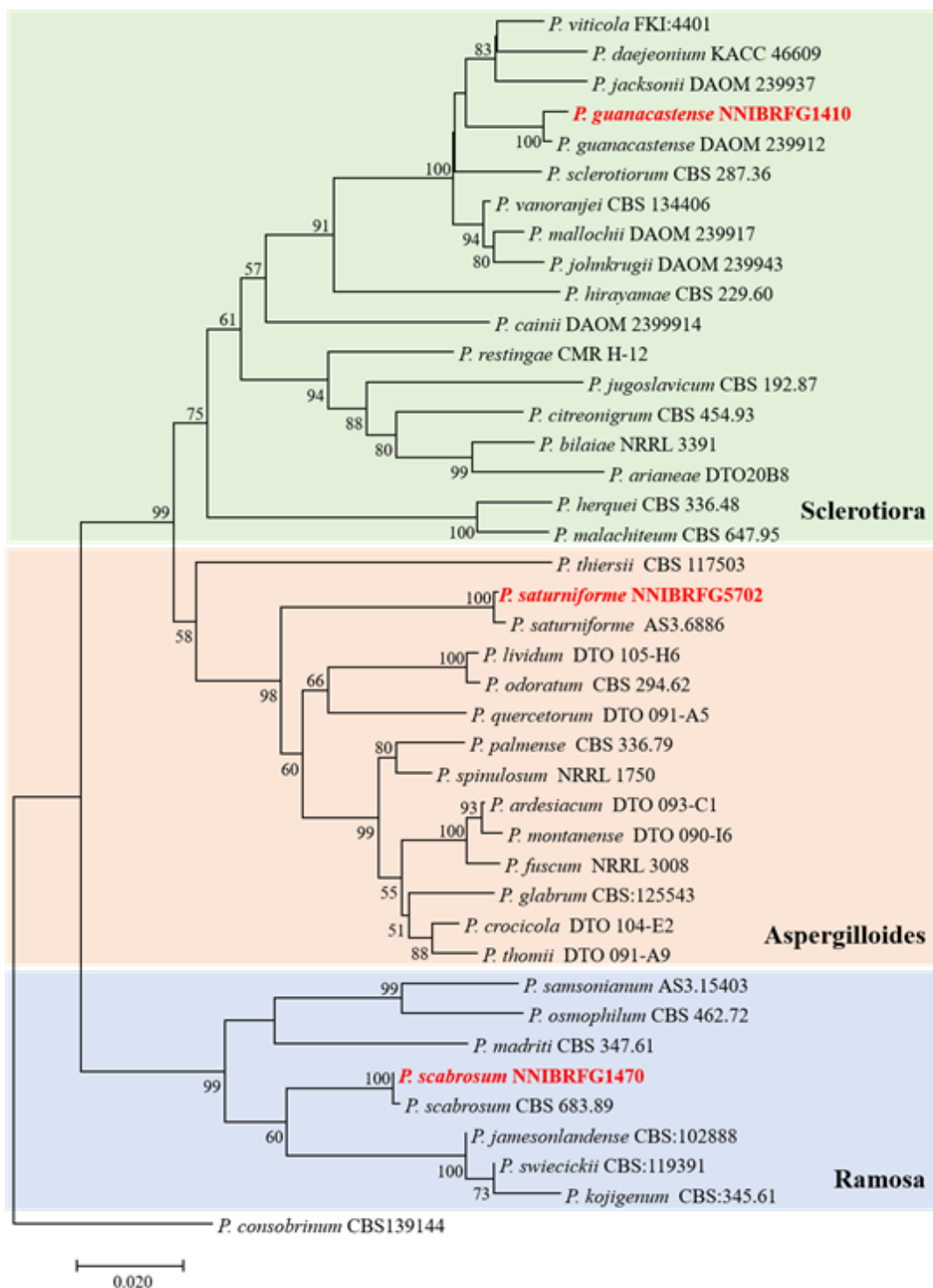
**The conidiophore** monoverticillate, smooth-walled, short stipes, hyaline; **phialide** 9.3-12  $\mu$ m length  $\times$  3.8-4.46  $\mu$ m width, cylindrical; **conidia** 2.77-4.47  $\mu$ m diameter, globose to subglobose.

**Phylogenetic section:** Sclerotiora

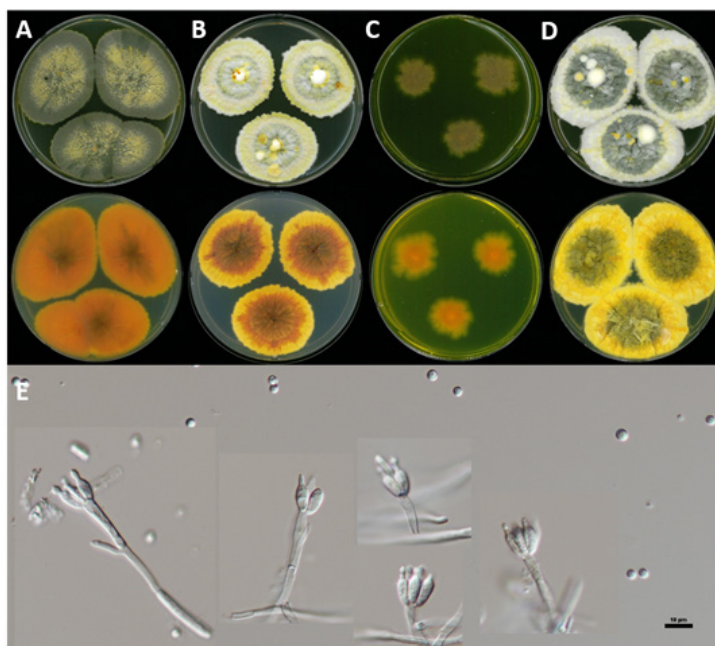
**Table 2.** GenBank accession numbers of isolates includes in this study.

Species	Strain	Genebank accession number		
		ITS	BT	CMD
<i>Penicillium ardesiacum</i>	DTO 093-C1	KM189565	KM088805	KM089190
<i>Penicillium arianae</i>	DTO20B8	KC773833	KC773784	KC773811
<i>Penicillium bilaiae</i>	NRRL 3391	JN714937	JN625966	KC773811
<i>Penicillium cainii</i>	DAOM 2399914	JN686435	KC773787	KC773814
<i>Penicillium citreonigrum</i>	CBS 454.93	AY157489	KC773790	KC773816
<i>Penicillium crocicola</i>	DTO 104-E2	KM189581	KJ834445	KM089210
<i>Penicillium daejeonium</i>	KACC 46609	JX436489	JX436493	JX436491
<i>Penicillium fuscum</i>	NRRL 3008	AF033411	GQ367513	GQ367539
<i>Penicillium glabrum</i>	CBS:125543	GU981567	GU981619	GQ367545
<i>Penicillium guanacastense</i>	DAOM 239912	JN626098	JN625967	JN626010
<i>Penicillium guanacastense</i>	NNIBRFG1410	MT995061		
<i>Penicillium herquei</i>	CBS 336.48	JN626101	JN625970	JN626013
<i>Penicillium hirayamae</i>	CBS 229.60	JN626095	JN625955	JN626003
<i>Penicillium jacksonii</i>	DAOM 239937	JN686437	JN686368	JN686391
<i>Penicillium jamesonlandense</i>	CBS 102888	NR_119570	KY989126	KJ866985
<i>Penicillium johnkrugii</i>	DAOM 239943	JN686447	JN686378	JN686401
<i>Penicillium jugoslavicum</i>	CBS 192.87	KC773836	KC773789	KC773815
<i>Penicillium kojigenum</i>	CBS 345.61	NR_121253	KJ834463	KJ867011
<i>Penicillium lividum</i>	DTO 105-H6	KM189582	KM088825	KM089211
<i>Penicillium madriti</i>	CBS 347.61	MH858079	KJ834470	EU644076
<i>Penicillium malachiteum</i>	CBS 647.95	KC773838	KC773794	KC773820
<i>Penicillium mallochii</i>	DAOM 239917	JN626104	JN625973	JN626016
<i>Penicillium montanense</i>	DTO 090-I6	KM189551	KM088789	KM089174
<i>Penicillium odoratum</i>	CBS 294.62	KC411730	KJ834478	KM089363
<i>Penicillium osmophilum</i>	CBS 462.72	MH860531	AY674376	KU896846
<i>Penicillium palmense</i>	CBS 336.79	KJ834515	GQ367508	GQ367534
<i>Penicillium quercetorum</i>	DTO 091-A5	KM189556	KM088795	KM089180
<i>Penicillium restingae</i>	CMR H-12	KF803355	KF803349	KF803352
<i>Penicillium samsonianum</i>	AS3.15403	KJ668590	KJ668582	KJ668586
<i>Penicillium saturniforme</i>	AS3.6886	EU644081	EU644080	EU644062
<i>Penicillium saturniforme</i>	NNIBRFG5702	MT995063		
<i>Penicillium scabrosum</i>	CBS 683.89	KM023349	KM023336	FJ530987
<i>Penicillium scabrosum</i>	NNIBRFG1470	MT995062		
<i>Penicillium sclerotiorum</i>	CBS 287.36	JN626132	JN626001	JN626044
<i>Penicillium spinulosum</i>	NRRL 1750	AF033410	KJ834493	GQ367524
<i>Penicillium swiecickii</i>	CBS 119391	NR_121254	KJ834494	KJ866993
<i>Penicillium thiersii</i>	CBS 117503	MH863022	KJ834497	AY741726
<i>Penicillium thomii</i>	DTO 091-A9	KM189560	KM088799	KM089184
<i>Penicillium vanoranjei</i>	CBS 134406	KC695696	KC695686	KC695691
<i>Penicillium viticola</i>	FKI 4401	AB606414	AB540174	AB540173

ITS: Internal transcribed spacer; BT:  $\beta$ -tubulin gene; CMD: Calmodulin gene



**Fig. 1.** Phylogenetic analysis using the neighbor-joining method based on internal transcribed spacer (ITS) region,  $\beta$ -tubulin and calmodulin gene sequences of three *Penicillium* species. *P. consobrinum* was used to an outgroup. Bootstrap values more than 50% (1,000 replications) are shown at branches. The new isolates from the present study are shown in bold and red.



**Fig. 2.** *Penicillium guanacastense* NNIBRFG1410. Colony shape on A, potato dextrose agar (PDA); B, Czapek yeast autolysate agar (CYA); C, creatine sucrose agar (CREA); D, yeast extract sucrose (YES); E, conidiophores and conidia. Scale bar is 10  $\mu$ m.

**Habitat:** Soil in stream

**Specimen examined:** Namsaengi-mot, Chocheon-uep, Jeju-do, Republic of Korea, 22 Mar 2016, NNIBRFG1410, Nakdonggang National Institute of Biological Resources

**Note:** NNIBRFG1410 is phylogenetically closely related to *P. guanacastense* within Sclerotiora clade. The molecular data of NNIBRFG1410 is identical to the strain of *P. guanacastense*, with high similarity in the ITS,  $\beta$ -tubulin and calmodulin sequence data, respectively. We confirm NNIBRFG1410 as *P. guanacastense* based on the morphology and phylogeny, and report the freshwater environment. The morphology of NNIBRFG1410 and Ref. species are similar conidiophore, phialide and conidia (Table 3).

**Table 3.** The morphology of *Penicillium* species.

Taxa	<i>Penicillium guanacastense</i>		<i>Penicillium saturniforme</i>		<i>Penicillium scabrosum</i>	
	NNIBRFG1410	Ref [7]	NNIBRFG5702	Ref [9]	NNIBRFG1470	Ref [10]
Conidiophore	monoverticillate, smooth-walled, short stipes, hyaline	monoverticillate, stipes septate, smooth to finely roughened,	mono or biverticillate, smooth-walled	mono or biverticillate, finely roughwalled, commonly with swollen apices	terverticillate, smooth-walled	bi- and terverticillate, a comparatively short
Rami					6.5–11.3 $\times$ 2.85–3.8 $\mu$ m	10-20 $\times$ 2.5-4.0 $\mu$ m
Metulae			10.15–13.18 $\times$ 3.3–3.79 $\mu$ m	10–20 $\times$ 3.5–4.5 (–5.4) $\mu$ m	6.19–8.6 $\times$ 2.65–3.3 $\mu$ m	15-25 $\times$ 2.5-4.0 $\mu$ m,
Phialide	9.3–12 $\times$ 3.8–4.46 $\mu$ m, cylindrical	7–10(–16) $\times$ 2–3(–4) $\mu$ m, ampulliform with a collula	6.52–7.23 $\times$ 3.03–3.49 $\mu$ m, cylindrical-shaped	7–11 $\times$ (2.0–)2.5–3.5(–4.0) $\mu$ m, ampulliform with short collula	7.6–9.4 $\times$ 2.3–2.6 $\mu$ m, flash-shaped	7-11 $\times$ 2.0-2.5 $\mu$ m, well-defined, abruptly narrowed collulum
Conidia	2.77–4.47 $\mu$ m diameter, globose to subglobose	2.5–3.5 $\times$ 2–2.5 $\mu$ m, globose to subglobose, finely roughened	3.19–3.94 $\mu$ m diameter, globose to subglobose	(3.0–)3.5–4.0 $\mu$ m, sphaeroidal, ovoid to ellipsoidal	6.19–8.6 $\mu$ m diameter, globose to subglobose	2.4-3.2 $\mu$ m, globose to subglobose, rough-walled



*Penicillium saturniforme* (L. Wang & W.Y. Zhuang) Houbraken & Samson, Studies in Mycology 70: 48 (2011)[8] (Fig. 1 and 3).

≡ *Eupenicillium saturniforme* L. Wang & W.Y. Zhuang, Mycopathologia 167 (6): 300 (2009).

**Mycobank No.:** MB541663

**Description:**

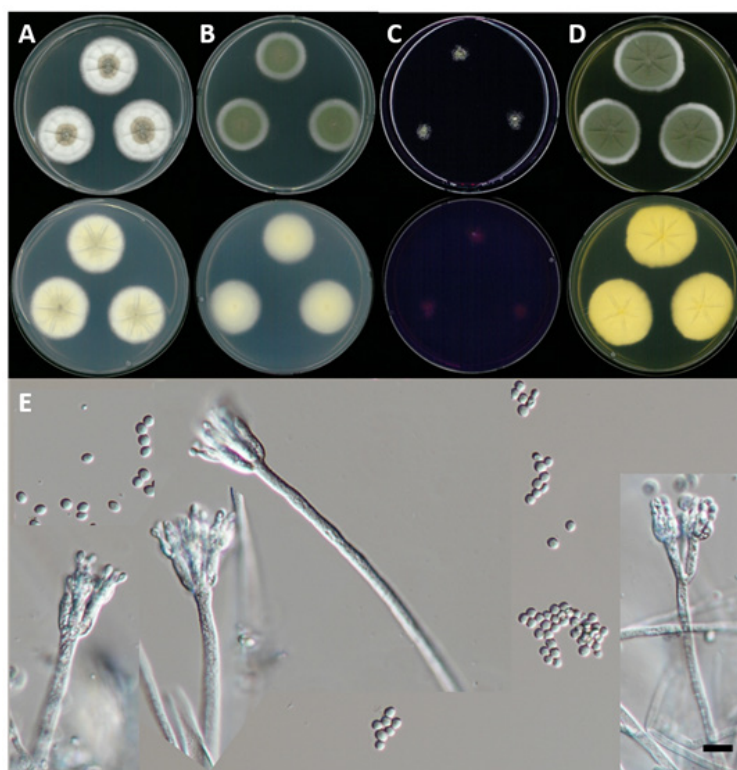
**Colonies on PDA** texture velutinous, radial slightly sulcate, front olive green to white, reverse pale yellow, 3 mm on 7 days at 25°C; **YES** texture floccose to fasciculate, slightly radial sulcate, front dark green to white, reverse light yellow, 3.5 mm on 7 days at 25°C; **CYA** texture floccose to granular, front dark green to white, reverse pale yellow, 2.5 mm on 7 days at 25°C

**The conidiophore** mono or biverticillate, smooth-walled; metulae 10.15-13.18  $\mu\text{m}$  length  $\times$  3.3-3.79  $\mu\text{m}$  width; **phialide** 6.52-7.23  $\mu\text{m}$  length  $\times$  3.03-3.49  $\mu\text{m}$  width, cylindrical-shaped; **conidia** 3.19-3.94  $\mu\text{m}$  diameter, globose to subglobose.

**Phylogenetic section:** Aspergilloides

**Habitat:** Soil in the river

**Specimen examined:** Soyang-river, Chuncheon-si, Gangwon-do, Republic of Korea, 03 May 2018, NNIBRFG5702, Nakdonggang National Institute of Biological Resources



**Fig. 3.** *Penicillium saturniforme* NNIBRFG5702. Colony shape on A, potato dextrose agar (PDA); B, Czapek yeast autolysate agar (CYA); C, creatine sucrose agar (CREA); D, yeast extract sucrose (YES); E, conidiophores and conidia. Scale bar is 10  $\mu\text{m}$ .

**Note:** NNIBRFG5702 is phylogenetically closely related to *P. saturniforme* within Aspergilloides clade. The molecular data of NNIBRFG5702 is identical to the strain of *P. saturniforme*, with high similarity in the ITS,  $\beta$ -tubulin and calmodulin sequence data, respectively. We confirm NNIBRFG5702 as *P. saturniforme* based on the morphology and phylogeny, and report the freshwater environment. The morphology of NNIBRFG5702 and Ref. species are similar conidiophore, metulae, phialide and conidia (Table 3).

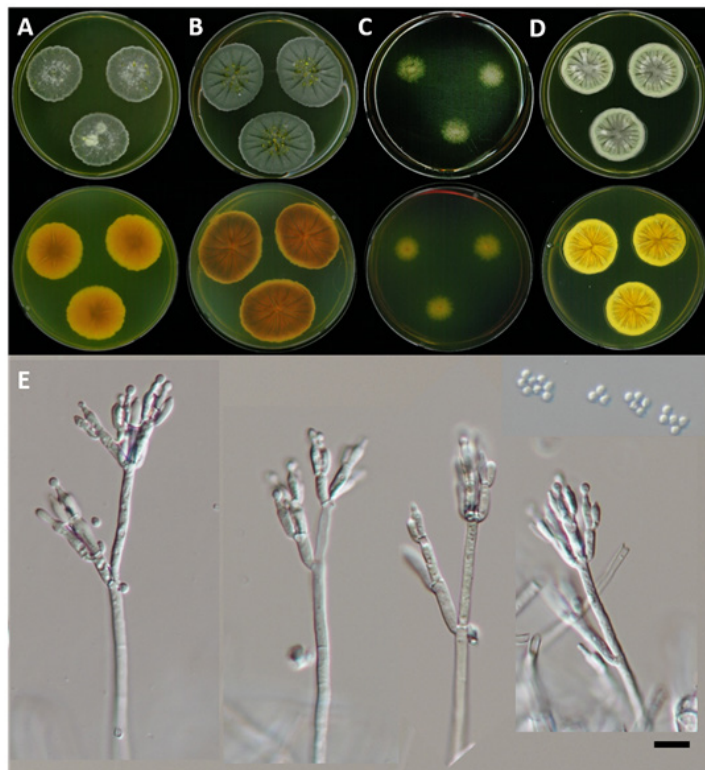
***Penicillium scabrosum*** Frisvad, Samson & Stolk, Persoonia 14 (2): 177 (1990)[9] (Fig. 1 and 4).

**Mycobank No.:** MB136735

**Description:**

**Colonies on PDA** texture floccose to fasciculate, radial sulcate, front pale green to dark green, reverse dark orange to light orange, 2.5 mm on 7 days at 25°C; **YES** texture floccose to granular, deeply radial sulcate, front grayish green to light moss green, reverse yellow, 2.5 mm on 7 days at 25°C; **CYA** texture floccose to granular, deeply radial sulcate, exudate pale yellow droplet, front light green, reverse dark grayish orange, 3.5 mm on 7 days at 25°C; **CREA** good acid production

**The conidiophore** terverticillate, smooth-walled; **Rami** 6.5-11.3  $\mu\text{m}$  length  $\times$  2.85-3.8  $\mu\text{m}$  width; **metulae** 6.19-8.6  $\mu\text{m}$  length  $\times$  2.65-3.3  $\mu\text{m}$  width; **phialide**, 7.6-9.4  $\mu\text{m}$  length  $\times$  2.3-2.6  $\mu\text{m}$  width, flask-shaped; **conidia** 6.19-8.6  $\mu\text{m}$  diameter, globose to subglobose.



**Fig. 4.** *Penicillium scabrosum* NNIBRFG1470. Colony shape on A, potato dextrose agar (PDA); B, Czapek yeast autolysate agar (CYA); C, creatine sucrose agar (CREA); D, yeast extract sucrose (YES); E, conidiophores and conidia. Scale bar is 10  $\mu\text{m}$ .

**Phylogenetic section:** *Ramosa*

**Habitat:** Soil in the stream

**Specimen examined:** Gamcheon, Gimcheon-si, Gyeongsangbuk-do, Republic of Korea, 23 Mar 2016, NNIBRFG1470, Nakdonggang National Institute of Biological Resources

**Note:** NNIBRFG1470 is phylogenetically closely related to *P. scabrosum* within *Ramosa* clade. The molecular data of NNIBRFG1470 is identical to the strain of *P. scabrosum*, with high similarity in the ITS,  $\beta$ -tubulin and calmodulin sequence data, respectively. We confirm NNIBRFG1470 as *P. scabrosum* based on the morphology and phylogeny, and report the freshwater environment. The morphology of NNIBRFG1470 and Ref. species are similar conidiophore, rami, matulae and phialade. Conidia size of NNIBRFG1470 are bigger than Ref. species (Table 3).

## ACKNOWLEDGEMENT

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